Evaluation of pseudothrombocytopenia causes

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ABSTRACT

Aim: In this single center study, subjects who had thrombocytopenia on the complete blood count were evaluated for pseudothrombocytopenia. We aim to evaluate the reasons of pseudothrombocytopenia who referred to outpatient hematology clinic for further investigation. Methods: A total of 220 patients who were referred to hematology outpatient clinics with low platelet counts (< 150x10⁹/L) were included in the study. 107 (48,6%) were female and 113 (51,4%) were male. Initially; the patients complete blood counts (CBC) were studied with ROCHE SYSMEX EX 2100 device. Then peripheric smears of these blood samples were reviewed by experienced hematologist to evaluate thrombocyte count and morphology. Pseudothrombocytopenia was defined if the thrombocyte count was found higher than CBC with peripheral smear. Results: In this study, we have demonstrated that 16,8% of the patients with thrombocytopenia (<150x10⁹/L) on complete blood count were pseudothrombocytopenic. The pseudothrombocytopenia rates were 4% (n=2), 7,2% (n=4) and 26,9% (n=31) if thrombocyte levels were less than 50x10⁹/L, between 50-100x10⁹/L and above 100x10⁹/L respectively. The causes of pseudothrombocytopenia as EDTA dependent trombocytopenia, giant trombocytes, Bernard Solier Syndrome, platelet clumps or platelet rosettes around white blood cells and inadequate blood sample. Conclusion: Diagnosis of thrombocytopenia without confirmation on the peripheral smear may expose patients to unnecessary diagnostic procedures and irrelevant therapies. During thrombocytopenia evaluation; peripheral smear examination should be performed as a first step to rule out the diseases such as EDTA dependent trombocytopenia, giant trombocytes and inherited trombocyte diseases that presented with pseudotrombocytopenia.

Keywords: Pseudothrombocytopenia, peripheral smear.

INTRODUCTION

The possibility of pseudothrombocytopenia (falsely low platelet count) should be eliminated before an extensive evaluation is undertaken. Pseudothrombocytopenia can occur in a number of settings, all of which can be identified by review of the peripheral blood smear and/or repeating the CBC using a non-EDTA anticoagulant. Incompletely mixed or inadequately anticoagulated samples may form a clot that traps platelets in the collection tube and prevents them from being counted. Giant platelets may be counted by automated counters as red blood cells rather than platelets. Giant platelets suggest a congenital platelet disorder (eg, May-Hegglin
anomaly, Bernard Soulier syndrome). Exposure of some patient samples to the EDTA anticoagulant in the collection tube can induce platelet clumps or platelet rosettes around white blood cells. These may be counted by automated counters as leukocytes rather than platelets. Approximately 0.1 percent of individuals have EDTA-dependent agglutinins that can induce platelet clumping. This is thought to result from a “naturally occurring” platelet autoantibody directed against a concealed epitope on platelet membrane glycoprotein (GP) IIb/IIIa that becomes exposed by EDTA-induced dissociation of GPIIb/IIIa (Bartels et al., 1997; Fiorin et al., 1998). Administration of GPIIb/IIIa inhibitors (eg, eptifibatide, tirofiban, abciximab) has also been implicated in this mechanism. On occasion, platelets may rosette around WBCs (eg, neutrophils, monocytes, lymphoma cells) (Bobba and Doll, 2012). This phenomenon has also been called “platelet satellitism.” In one case, this resulted from the presence of an EDTA-dependent antibody with dual reactivity against GP IIb/IIIa and the neutrophil Fc gamma receptor III (Podda et al., 2012). If platelet clumping is observed, the platelet count is repeated using heparin or sodium citrate as an anticoagulant in the collection tube. If citrate is used, the platelet count should be corrected for dilution caused by the amount of citrate solution; no such correction is needed for heparin. Alternatively, fresh, non-anticoagulated blood can be pipetted directly into platelet-counting diluent fluid. Inability to notice pseudothrombocytopenia may result in misdiagnoses and mistreatments. Thus, evaluation of the peripheral smear has utmost importance. In this single center study, subjects who had thrombocytopenia on the complete blood count were evaluated for pseudothrombocytopenia.

METHODS

A total of 220 patients who were referred to hematology out-patient clinics between January-December 2010 because of having low platelet counts (<150x10^9/L) were included in the study. The age of the patients ranged between 17 and 82 and the mean age was 45.16±17.76; 107 (48.6%) was female and 113 (51.4%) was male. Complete blood count of patients was studied ROCHE SYSMEX EX 2100 model device. The platelet counts and morphologies were evaluated with Giemsa stained peripheral smears in cases with thrombocyte count less than 150x10^9/L. The smears were examined under light microscope by hematology team including 3 hematologist and 2 internal medicine residents. The mean platelet counts from 10 hpf was calculated and multiplied by 10000. Additionally, in order to determine the range of platelet count at which the counter produces the highest error, the patients were subclassified into three groups according to the CBC-based platelet count; group 1= <50x10^9/L, group 2= 50-100x10^9/L and group 3=100-149x10^9/L.

RESULTS

The mean platelet count was 87.9 ± 42.5x10^9/L (Range: 3-147x10^9/L). In this study, we have demonstrated that 16.8% of the patients who had thrombocytopenia (<150x10^9/L) on complete blood count had pseudothrombocytopenia. The pseudothrombocytopenia rates were 4% (n=2), 7.2% (n=4), 5.7% (n=6) and 26.9% (n=31) if thrombocyte levels were less than 50x10^9/L, between 50-100x10^9/L, between 100-1000x10^9/L, less than 100x10^9/L and above 100x10^9/L respectively. The causes of pseudothrombocytopenia were EDTA dependent trombocytopenia (n=1), giant trombocytes (n=3), Bernard Soulier Syndrome (n=1), platelet clumps around white blood cells (n=1) and inadequate blood sample (n=15). The peripheral smear pictures of large platelets, giant platelets and EDTA dependent pseudotrombocytopenia were presented (peripheral 1,2,3).
DISCUSSION

In routine laboratories for blood cell counts are evaluated with automated blood counter machines. The number of thrombocytes were measured in EDTA- or CPT-anticoagulated blood samples immediately after sampling. Although the results are accurate and sensitive, there might be false negative results by 0.1-0.2%. Cold agglutinins, paraproteins, contact with foreign surfaces such as dialysis membrane, giant platelets, platelet satellite, hyperlipidemia or EDTA dependent platelet clumping might cause false negative results. Peripheric smear should be performed to rule out these causes. The accurate number of trombocytes and morphologic abnormalities would be detected with this evaluation.

Diagnosis of thrombocytopenia without confirmation on the peripheral smear may expose patients to unnecessary diagnostic procedures and irrelevant therapies (Bizzaro, 1995; Froom and Barak, 2011; Gschwandtner et al., 1997; Bartels et al., 1997; Oliveira et al., 2003; Kunz, 2001). A study on 217 cases revealed that 10% of the cases with pseudothrombocytopenia were administered irrelevant treatments (Szczepinski et al., 2009). In one case report, after trombocyte count was found 8x10^9/L before pericardiosynthesis, trombocyte replacement and bone marrow analysis was planned. But detecting clusters on peripheric smear has prevented all
these unnecessary and invasive interventions. To avoid these, it is imperative that a peripheral smear be examined and a complete blood count repeated using a different anticoagulant in an asymptomatic patient who gives unexpected low PLT counts, as illustrated in this case (Lau et al., 2004). In another case who underwent splenectomy operation due to immune thrombocytopenic purpura had low platelet count (35x10^9/L) during operation. CBC was repeated with citrate coated tube and platelet count was found 138x10^9/L (Yamada et al., 2008).

In this study, the frequency of pseudothrombocytopenia among cases with platelet count less than 150x10^9/L have been found as 16.8%. The pseudothrombocytopenia rates were 4% (n=2), 7.2% (n=4) and 26.9% (n=31) if thrombocyte levels were less than 50x10^9/L, between 50-10010^9/L and above 100x10^9/L respectively. The causes of pseudothrombocytopenia were EDTA dependent trombocytopenia (n=1), giant trombocytes (n=3), Bernard Solier Syndrome (n=1), platelet clumps around white blood cells (n=1) and inadequate blood sample (n=15).

In other studies, the frequency of pseudothrombocytopenia in all patients admitted to hospitals and in patients with isolated thrombocytopenia have been reported as 0.09-0.21% and 15-30% respectively. The frequency of pseudothrombocytopenia in patients who had CBC based platelet count <100x10^9/L was 5.7% and this result is compatible with the literature (Froom and Barak, 2011; Szczepinski et al., 2009; Cohen et al., 2000; Silvestri et al., 1995).

Our limitations are small patient population, platelet count was 100-149x10^9/L in the range half of the total number of cases and erroneous measurement of the blood counter. Our strengths are peripheral smears were discussed and evaluated by an experienced team including hematologist at the same time. All trombocytopenic cases were confirmed with both hemogram and peripheral smear.

CONCLUSION

Thrombocytopenia is defined as a platelet count of less than 150x10^9/L. It is often discovered incidentally when obtaining a complete blood count during an office visit. The etiology usually is not obvious, and additional investigation is required. In this study we evaluated rare pseudothrombocytopenia reasons and more importantly we want to emphasize the importance of peripheral smear during thrombocytopenia evaluation. Diagnosis of thrombocytopenia without confirmation on the peripheral smear may expose patients to unnecessary diagnostic procedures and irrelevant therapies.

REFERENCES


